Correspondence

An improved low-formaldehyde embalming fluid to preserve cadavers for anatomy teaching

The desired properties required for successful embalming of cadavers for gross anatomy teaching include: (1) good long-term structural preservation of organs and tissues with minimal shrinkage or distortion; (2) prevention of over-hardening, while maintaining flexibility and suppleness of internal organs; (3) prevention of desiccation; (4) prevention of fungal or bacterial growth and spread within a specific cadaver and to other cadavers in the dissection room; (5) reduction of potential biohazards (spread of infection to dissection personnel and students); (6) reduction of environmental chemical hazards (especially from formaldehyde and phenol) in order to comply with increasingly severe health and safety regulations and a new awareness of possible dangers of these chemicals in the workplace; and (7) retention of colour of tissues and organs while minimising oxidation effects that result in 'browning'.

The potential health and safety problems for staff and students in gross anatomy laboratories and the need to comply with increasingly restrictive exposure limits to components of embalming chemicals, especially formaldehyde, have caused us to try and find practical solutions from 2 directions. We have introduced a novel type of dissection 'bed' with an internal motor that causes a downflow of formaldehyde-rich vapours, which is absorbed by a replaceable active carbon filtration system (Coleman, 1995). In addition we have experimented with our embalming fluid in order to reduce the percentage of formaldehyde. We have introduced a new embalming mixture with a relatively low formaldehyde content, but with a very high salt content. Our new embalming mixture results in cadavers with excellent dissection properties and a dissection room that is virtually free of smell and which complies with the most severe environmental safety restrictions. In order to confirm the efficacy of the embalming, necropsy samples of a wide range of tissue and organs were taken for histological examination.

All the cadavers are from persons who voluntarily donated their bodies to science. The cadavers reach the mortuary within 24 h of death and are kept refrigerated at 4 °C until embalming, which is usually within 1–3 d. Our embalming solution consists of 37–40% formaldehyde (0.5 l), phenol (0.2 l), glycerine (0.5 l), isopropyl alcohol (4 l) and sodium chloride (20 kg) made up to approximately 35 l final volume with tap water. With small cadavers we use only 25 l final volume. The final concentration of formaldehyde in the embalming mixture is only 0.5–0.75%. The chemicals used are all technical grade. The sodium chloride is common household salt purchased from a local supermarket. This refined table salt dissolves more easily than coarse rock salt. The embalming fluid is perfused via the femoral artery using a mechanical pressure pump at 750–1000 mmHg. The cadavers are then closed in thick polyethylene sheeting and stored in this embalming solution at 18 °C for at least 3 mo, although preferably for up to 1 y or longer, before dissection. Before opening the bodies for dissection the excess embalming fluid is removed and the cadavers are left fairly dry. This is essential using our new dissection 'beds'. The high salt content retained in the tissues prevents any further significant desiccation.

Formaldehyde vapour levels in the anatomy laboratory are determined by an approved external independent assessor to ensure that we comply with the legal limitations for exposure to formaldehyde in the workplace. We measure formaldehyde levels on the first day of student dissection, when the cadavers are first opened and when exposure to formaldehyde vapour is at its greatest. This is to ensure that we do not exceed the permitted ceiling values.

All necropsies were from bodies aged 85–94 y at death. Following several months repeated exposure of the cadavers during dissection classes and prior to preparation for burial (typically 12–15 mo postmortem), blocks of tissues and organs were taken for histological examination. The tissues were placed in 70% ethanol, dehydrated in 95% and absolute ethanol and embedded for routine histology in paraffin wax. Microtomy sections (6 μm) were stained by haematoxylin and eosin (H & E). Cartilage-containing sections (trachea, thyroid cartilage) were stained for metachromasia in 0.1% toluidine blue in 1% sodium tetraborate (borax) and by the periodic acid–Schiff (PAS) technique. Bone samples were treated with Rapid Bone Decalcifier (Eurobio, Les Ulis Cedex B, France) for 48 h prior to wax embedding. Photomicrographs were taken using a Zeiss Axiophot microscope. Muscle and bone samples were also examined using polarisation microscopy.

The preservative properties of the embalming solution proved to be excellent. The gross anatomy of tissues and organs showed minimal structural distortion and the tissues remained supple and easy to dissect. There was very little desiccation and it was not necessary to add any additional fluid. The tissues retained much of their natural colour, and there was no indication, even over the long term, of 'browning' oxidation effects. Tissues that are normally quite sensitive to poor embalming, such as brain, retained their light colouration. There was no indication of any fungal growth whatsoever. In some cases there were minor salt deposits on the skin or internal organs.

The histology of the necropsy samples confirmed the excellence of the fixation in a wide range of tissues and organs (Fig.). Necropsies included striated, cardiac and smooth muscle, adipose tissue, liver, kidneys, lungs, trachea, brain, spinal cord, peripheral nerves, pancreas, thyroid glands, suprarenal glands, uterus, clitoris and bone (long bones, flat bones, vertebrae). The tissues showed minimal shrinkage and none of the shrinkage artefacts commonly

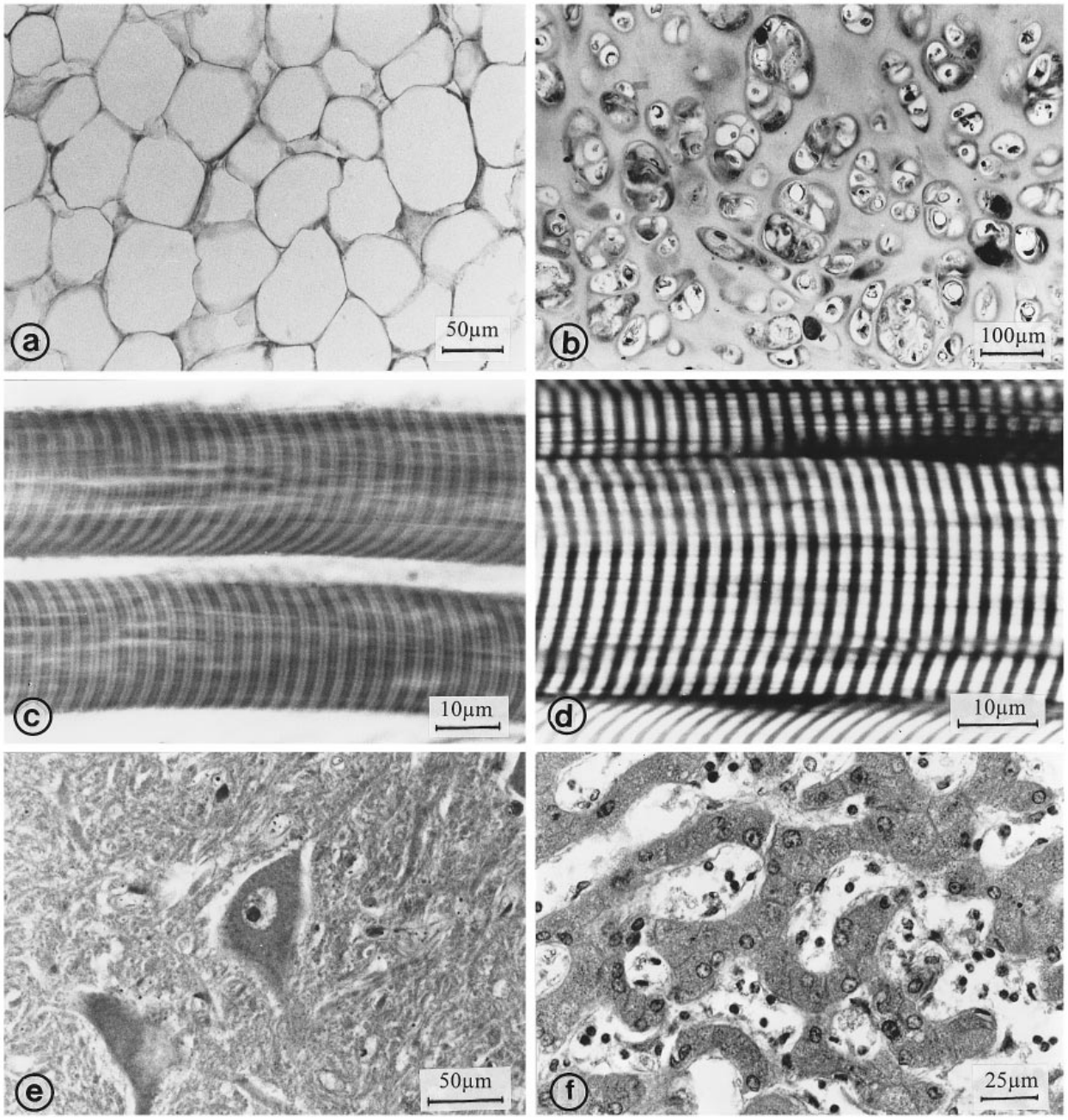

Fig. Necropsy samples showing the excellent long-term microanatomical preservation of various tissues from cadavers prepared with our novel embalming solution. (*a*) Adipose tissue showing no shrinkage artefacts (H & E). (*b*) Tracheal cartilage with metachromatic staining of matrix (toluidine blue staining). (*c*) Striated muscle fibres (tibialis anterior) showing well-preserved sarcomeric banding (H & E). (*d*) Striated muscle (as in *c*) viewed by polarisation microscopy showing preservation of anisotropic (A) banding. (*e*) Spinal cord (grey matter) thoracic segment illustrating excellent preservation of motor neuron and neuropil (toluidine blue staining). (*f*) Liver showing well-preserved hepatocytes and sinusoids (H & E).

seen in necropsies (see Fig.). Lipofuscin was readily seen in skeletal muscle, cardiac muscle, liver, brain and spinal cord. A spontaneous chromaffin reaction was sometimes observed in secretory cells of the suprarenal medulla. The intestinal mucosa was relatively poorly preserved, probably owing to autolytic postmortem effects that had occurred before embalming. The matrix of tracheal cartilage showed strong metachromasia and PAS-positive staining indicating that the matrix components were well preserved. The goblet cells of the tracheal mucosa and mucous cells of the tracheal glands were also strongly stained with PAS. Moreover, the mucus layer lining the pseudostratified epithelium of the trachea was retained and contained entrapped bacteria. This mucus layer stained positively with the PAS reaction.

In recent years there has been an increasing awareness of the potential health hazards of formaldehyde exposure in

the workplace (Coleman, 1995). The introduction of new standards restricting levels of exposure to formaldehyde has resulted in the need to try and find practical solutions to comply with health and safety regulations or face closure of gross anatomy laboratories. University authorities, Safety Officers and Medical Faculties are being faced with potential major litigation in the event that workers are exposed to formaldehyde levels above the legal limits or believe their personal safety is compromised. Classical embalming mixtures, used for decades, are now impractical. The search for newer low-formaldehyde embalming solutions or those with formaldehyde-substitutes has become an urgent issue. However, the subject has been largely neglected and relatively few reports on embalming of cadavers for gross anatomy laboratories have appeared or addressed this issue (Bradbury & Hoshino, 1978; Logan, 1983; Frolich et al. 1984; Wineski & English, 1989; O'Sullivan & Mitchell, 1993; Macdonald & MacGregor, 1997). Most of the reports deal with the need to reduce the concentration of the formaldehyde in embalming fluids or the use of formaldehyde substitutes.

We have had excellent results in reducing formaldehyde levels in our gross anatomy laboratory since the introduction of our new dissection 'beds' (Coleman, 1995). This system enables reduction of formaldehyde levels to within the local legal limits. In order to try and reduce the exposure levels of formaldehyde even further, we have experimented with the composition of our embalming solutions and have shown that 0.5 l 37–40 % formaldehyde per body is adequate. (This is a 50 % reduction on our 'classical' embalming solution.)

Formaldehyde levels on the day in which the cadavers were first opened, when values are at their greatest, were extremely low. Both personal samples (detectors on student laboratory coat lapels) and area samples were typically less than 10 % of permitted values of 0.37 ppm. Even 20 cm above the opened thoracic region of the cadavers, the formaldehyde levels did not exceed the legal limits. There was very little smell of formaldehyde in the dissection room. The extremely low formaldehyde levels in the laboratory, partly the result of the introduction of the dissection beds and partly the result of reduced levels in the embalming solution allowed prolonged dissection by staff and students without any of the common complaints associated with formaldehyde exposure such as eye-watering, or disturbances to the respiratory tract.

We have shown that our embalming solution with high salt levels results in excellent long-term preservation of cadavers and this has been confirmed by the histology of the necropsies seen in a wide range of tissues and organs. Salt has been used for centuries as a cheap and excellent preservative in food technology. High salt levels are common in many processed foods, such as meat, fish and canned vegetables. The high salt content of foodstuffs typically provides environments that minimise bacterial and fungal spoilage. As far as we are aware this is the first report in modern times of the use of high salt levels in preservation of cadavers.

The idea to use common salt as a major component in the embalming solution derived from its widespread use over the centuries as a food preservative, especially in meat. The skeletal muscles ('meat') of the human body comprise some 40–50 % of the body weight. Salted or cured meat remains soft and has fairly good resistance to desiccation and microbial spoilage. Moreover, common salt is readily available and also very cheap. We have found that fine-grain, free-flowing, supermarket salt is more convenient to use as it dissolves more easily than bulk rock salt in the embalming solution. We do not know the mechanics of the preservation process but believe that the salt provides chemical properties to the embalming solution similar to those of pickled or processed foods with high-salt content. Based on the excellent histology of the necropsies, even following prolonged postmortem periods, we believe that the salt may enhance the fixation properties of formaldehyde. Formol saline fixative, which was once fairly commonly used as a routine histological fixative, contains 0.9 % sodium chloride; in our embalming mixture the salt levels are considerably greater.

Embalming of the dead has been known from ancient times (Carter, 1972) and salt appears to have been used in the embalming process. The Egyptians are known to have successfully embalmed bodies and the earliest Biblical texts refer to the practice. 'Joseph commanded his servants the physicians to embalm his father: and the physicians embalmed Israel. And forty days were fulfilled for him: for so are fulfilled the days of those which are embalmed: and the Egyptians mourned for him three score and ten days…' (Genesis, chapter 50, vv 2, 3). Herodotus, the great Greek historian, writing some 9 centuries after the death of Tutankhamen, gives an account of Egyptian embalming. 'After treating the brain and soft body parts… the body is placed in Natrum for seventy days and entirely covered' (Carter, 1972). Howard Carter in 1925 reporting on the examination of the mummy of Tutankhamen wrote '… Mr Lucas examined some whitish spots on the skin… and these proved to be composed of 'common salt with a small admixture of sodium sulphate' in all probability derived from the natron used in the embalming process' (Carter, 1972). Thus the use of salt in embalming has an ancient history.

We believe that our simple modification of the embalming mixture with its high salt component and relatively low formaldehyde content provides an excellent method for embalming cadavers for anatomy teaching. Moreover when combined with techniques for absorbing formaldehyde vapour, the formaldehyde levels of the anatomy laboratory are so low that even the most severe environmental restrictions are met. Our method has improved the safety and working environment in the anatomy teaching laboratory. We have also proved the efficacy of the long-term embalming process by the necropsy sampling.

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